FIG. 1a(1)

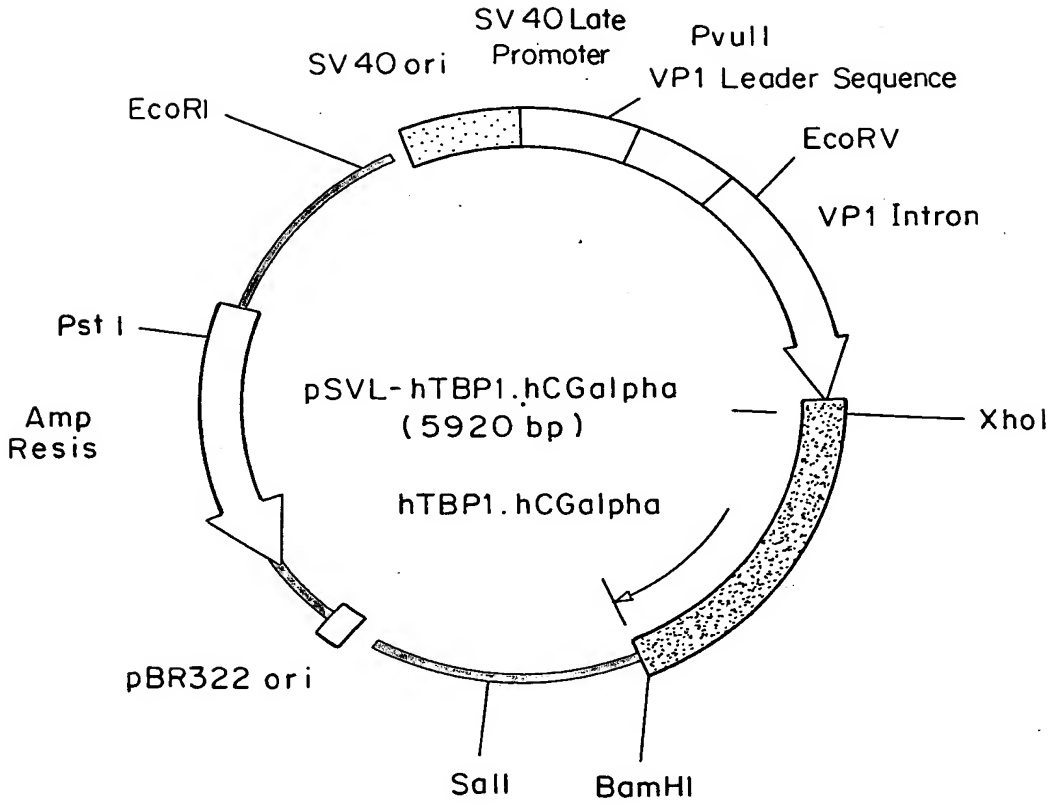

SV 40 Late
Promoter
Pvull
SV 40 ori
VP1 Leader Sequence
EcoRI
EcoRV
VP1 Intron
Pst I
pSVL-hTBP1.hCGalpha
(5920 bp)
Amp
Resis
XhoI
hTBP1.hCGalpha
pBR322 ori
SalI
BamHI

FIG. 1a(2)

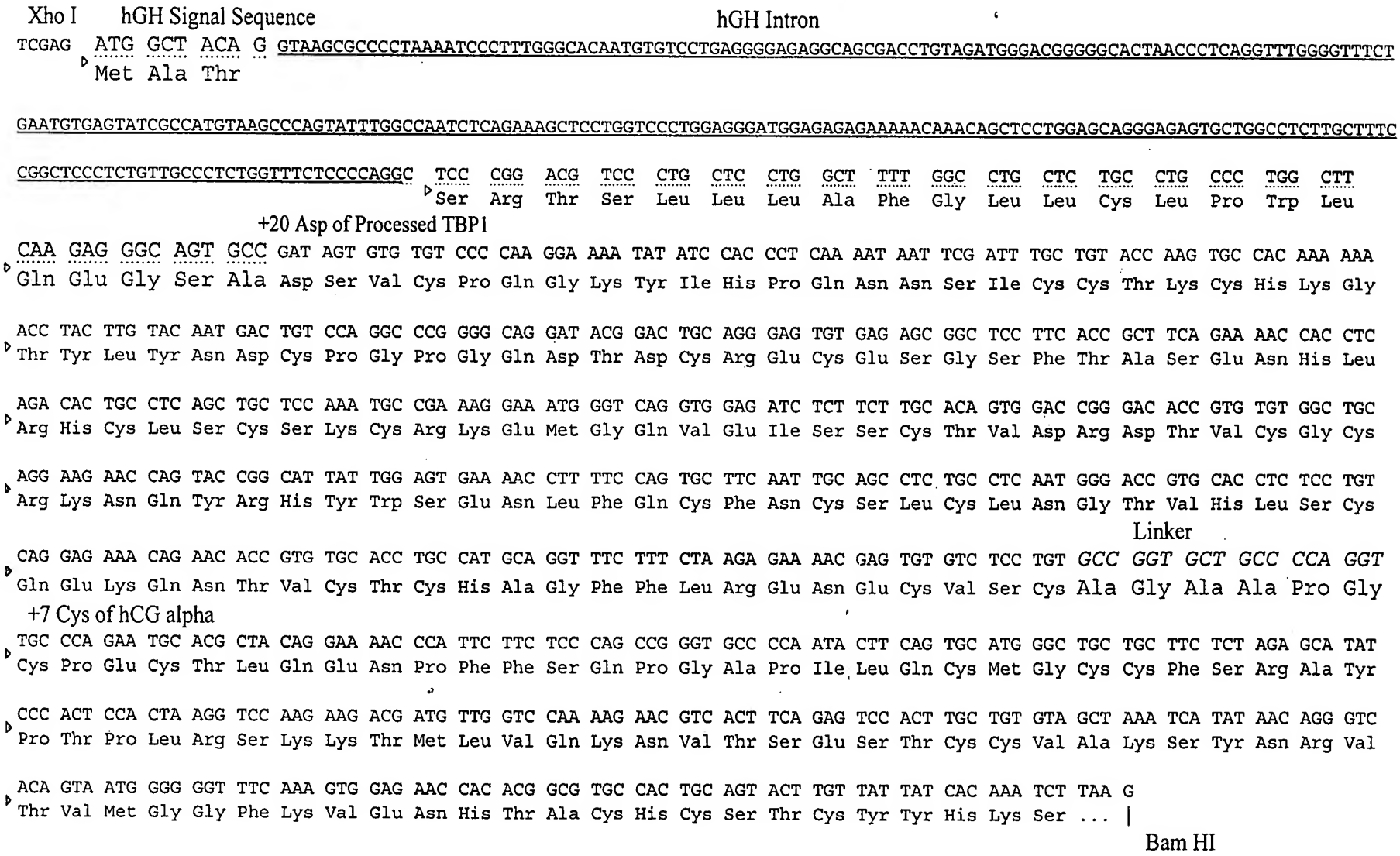

Xho I
hGH Signal Sequence
hGH Intron
TCGAG ATG GCT ACA G GTAAGCGCCCCTAAAATCCCTTTGGGCACAATGTGTCCTGAGGGGAGAGGCAGCGACCTGTAGATGGGACGGGGGCACTAACCCTCAGGTTTGGGGTTTCT
Met Ala Thr
GAATGTGAGTATCGCCATGTAAGCCCAGTATTTGGCCAATCTCAGAAAGCTCCTGGTCCCTGGAGGGATGGAGAGAGAAAAACAAACAGCTCCTGGAGCAGGGAGAGTGCTGGCCTCTTGCTTTC
CGGCTCCCTCTGTTGCCCTCTGGTTTCTCCCCAGGC TCC CGG ACG TCC CTG CTC CTG GCT TTT GGC CTG CTC TGC CTG CCC TGG CTT
Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu Cys Leu Pro Trp Leu
+20 Asp of Processed TBP1
CAA GAG GGC AGT GCC GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT TCG ATT TGC TGT ACC AAG TGC CAC AAA AAA
Gln Glu Gly Ser Ala Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys Gly
ACC TAC TTG TAC AAT GAC TGT CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TGT GAG AGC GGC TCC TTC ACC GCT TCA GAA AAC CAC CTC
Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
AGA CAC TGC CTC AGC TGC TCC AAA TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TCT TGC ACA GTG GAC CGG GAC ACC GTG TGT GGC TGC
Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys
AGG AAG AAC CAG TAC CGG CAT TAT TGG AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC AAT GGG ACC GTG CAC CTC TCC TGT
Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys
Linker
CAG GAG AAA CAG AAC ACC GTG TGC ACC TGC CAT GCA GGT TTC TTT CTA AGA GAA AAC GAG TGT GTC TCC TGT GCC GGT GCT GCC CCA GGT
Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ala Gly Ala Ala Pro Gly
+7 Cys of hCG alpha
TGC CCA GAA TGC ACG CTA CAG GAA AAC CCA TTC TTC TCC CAG CCG GGT GCC CCA ATA CTT CAG TGC ATG GGC TGC TGC TTC TCT AGA GCA TAT
Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys Phe Ser Arg Ala Tyr
CCC ACT CCA CTA AGG TCC AAG AAG ACG ATG TTG GTC CAA AAG AAC GTC ACT TCA GAG TCC ACT TGC TGT GTA GCT AAA TCA TAT AAC AGG GTC
Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser Tyr Asn Arg Val
ACA GTA ATG GGG GGT TTC AAA GTG GAG AAC CAC ACG GCG TGC CAC TGC AGT ACT TGT TAT TAT CAC AAA TCT TAA G
Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser ... |
Bam HI

FIG. 1b(1)

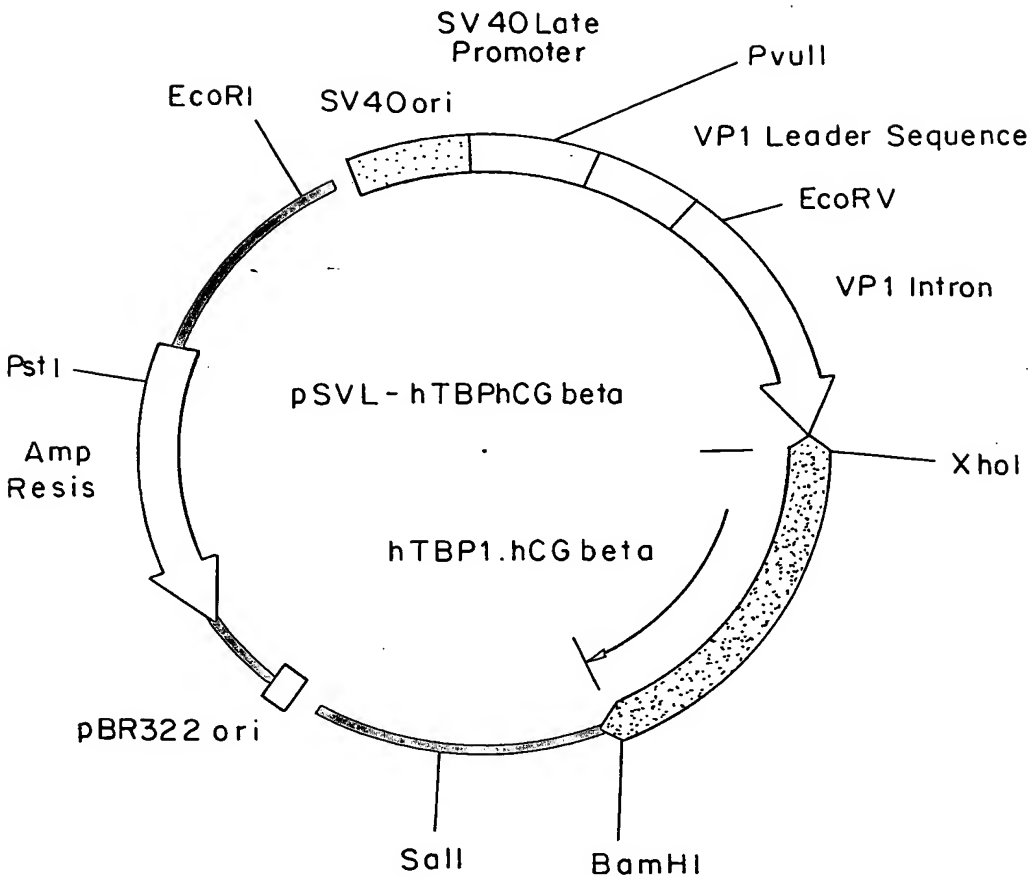

SV40 Late
Promoter
Pvull
EcoRI
SV40ori
VP1 Leader Sequence
EcoRV
VP1 Intron
PstI
pSVL - hTBPhCG beta
Amp
Resis
XhoI
hTBP1.hCG beta
pBR322 ori
SalI
BamHI

FIG. 1b(2)

hGH Signal Sequence hGH Intron

XhoI

CTCGAG ATG GCT ACA G GTAAGCGCCCCTAAAATCCCTTTGGGCACAATGTGTCCTGAGGGGAGAGGTAGCGACCTGTAGATGGGACGGGGGCACTAACCCTCAGGTTTGGG

Met Ala Thr

GCTTCTGAATGTGAGTATCGCCATGTAAGCCCAGTATTTGGCCAATCTCAGAAAGCTCCTGGTCCCTGGAGGGATGGAGAGAGAAAAACAAACAGCTCCTGGAGCAGGGAGAGTGCTGAC

CTCTTGCTCTCCGGCTCCCTCTGTTGCCCTCTGGTTTCTCCCCAGGC TCC CGG ACG TCC CTG CTC CTG GCT TTT GGC CTG CTC TGC CTG

Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu Cys Leu

+20 Asp of Processed TBP1

CCC TGG CTT CAA GAG GGC AGT GCC GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT TCG ATT TGC TGT ACC

Pro Trp Leu Gln Glu Gly Ser Ala Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr

AAG TGC CAC AAA GGA ACC TAC TTG TAC AAT GAC TGT CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TGT GAG AGC GGC TCC TTC ACC

Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr

GCT TCA GAA AAC CAC CTC AGA CAC TGC CTC AGC TGC TCC AAA TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TCT TGC ACA GTG GAC

Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp

CGG GAC ACC GTG TGT GGC TGC AGG AAG AAC CAG TAC CGG CAT TAT TGG AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC

Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu

AAT GGG ACC GTG CAC CTC TCC TGC CAG GAG AAA CAG AAC ACC GTG TGC ACC TGC CAT GCA GGT TTC TTT CTA AGA GAA AAT GAG TGT GTC

Asn Gly Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val

Linker +7 Pro of hCG beta

TCC TGT GCT GGT GCT GGT CCA CGG TGC CGC CCC ATC AAT GCC ACC CTG GCT GTG GAG AAG GAG GGC TGC CCC GTG TGC ATC ACC GTC

Ser Cys Ala Gly Ala Gly Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val

AAC ACC AAC ATC TGT GCC GGC TAC TGC CCC ACC ATG ACC CGC GTG CTG CAG GGG GTC CTG CCG GCC CTG CCT CAG GTG GTG TGC AAC TAC

Asn Thr Thr Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr

CGC GAT GTG CGC TTC GAG TCC ATC CGG CTC CCT GGC TGC CCG CGC GGC GTG AAC CCC GTG GTC TCC TAC GCC GTG GCT CTC AGC TGT CAA

Arg Asp Val Arg Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln

TGT GCA CTC TGC CGC CGC AGC ACC ACT GAC TGC GGG GGT CCC AAG GAC CAC CCC TTG ACC TGT GAT GAC CCC CGC TTC CAG GAC TCC TCT

Cys Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser

TCC TCA AGG GCC CCT CCC CCC AGC CTT CCA AGC CCA TCC CGA CTC CCG GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA TAA

Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln ***

Bam HI

FIG. 2a(1)

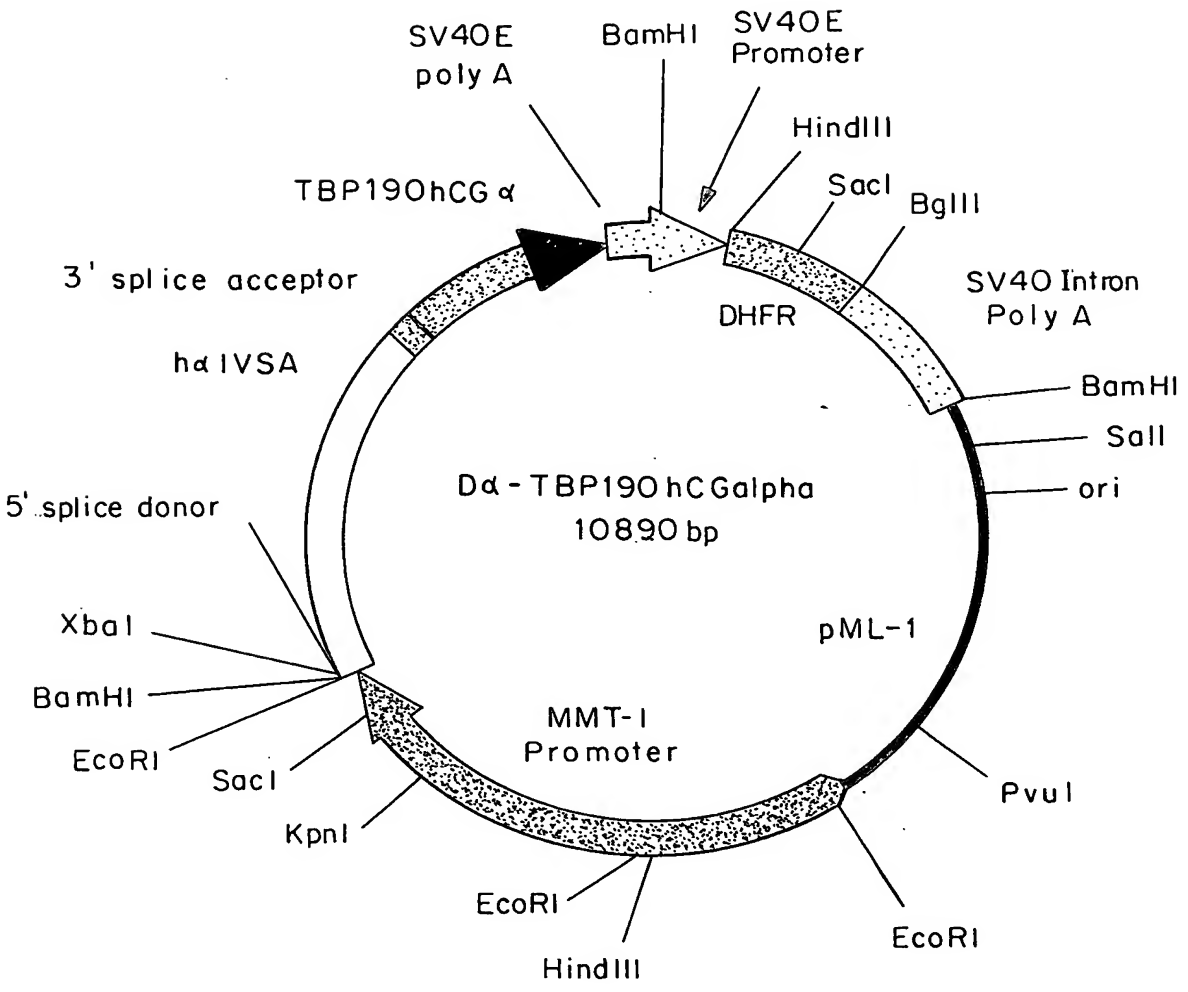

SV40E
poly A
BamHI
SV40E
Promoter
HindIII
SacI
BglII
TBP190hCG α
3' splice acceptor
hα IVSA
DHFR
SV40 Intron
Poly A
BamHI
SalI
ori
Dα - TBP190 hCGalpha
10890 bp
5' splice donor
Xbal
BamHI
EcoRI
SacI
KpnI
pML-1
MMT-I
Promoter
PvuI
EcoRI
EcoRI
HindIII

FIG. 2a(2)

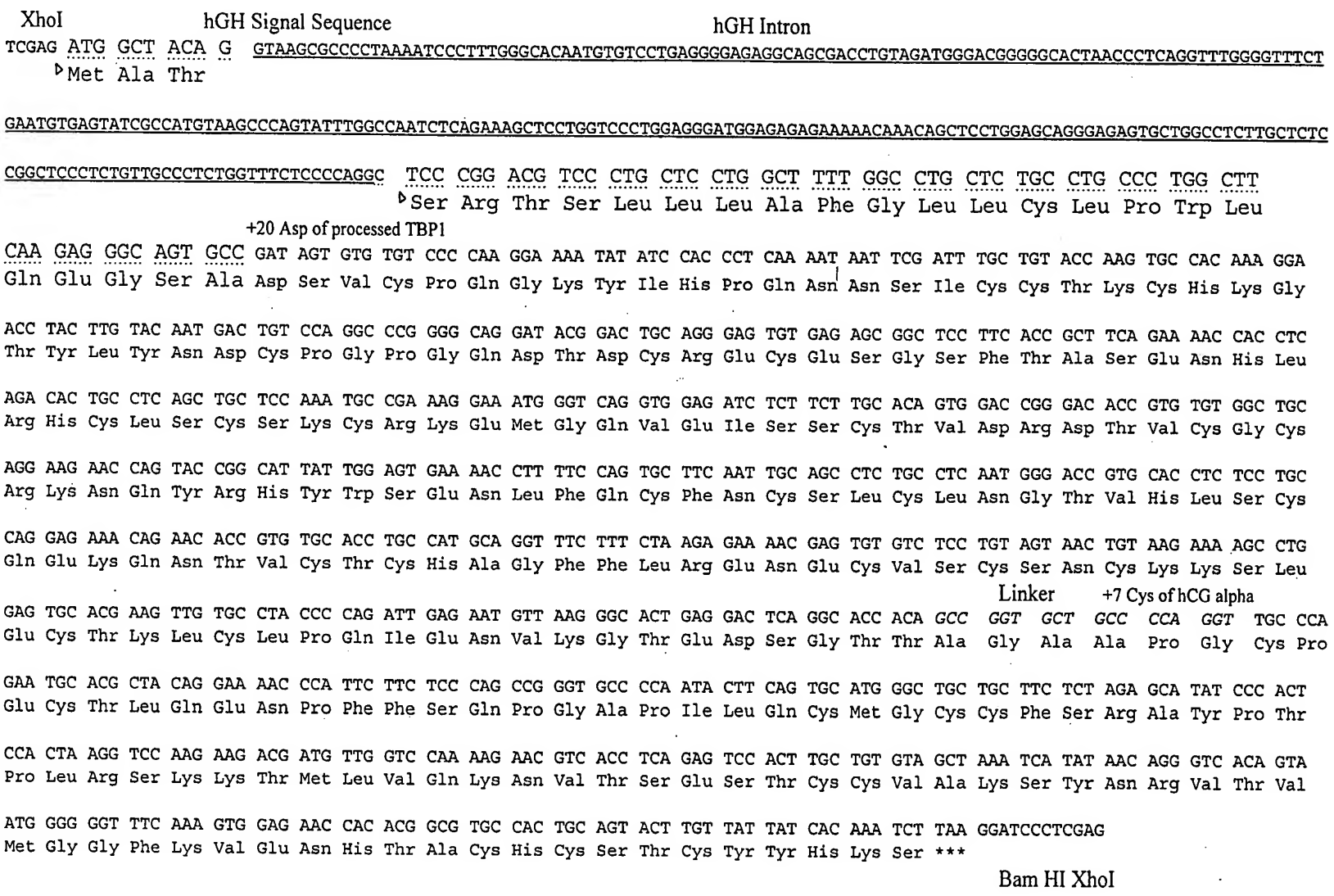

XhoI
hGH Signal Sequence
hGH Intron
TCGAG ATG GCT ACA G GTAAGCGCCCCTAAAATCCCTTTGGGCACAATGTGTCCTGAGGGGAGAGGCAGCGACCTGTAGATGGGACGGGGGCACTAACCCTCAGGTTTGGGGTTTCT
Met Ala Thr
GAATGTGAGTATCGCCATGTAAGCCCAGTATTTGGCCAATCTCAGAAAGCTCCTGGTCCCTGGAGGGATGGAGAGAGAAAAACAAACAGCTCCTGGAGCAGGGAGAGTGCTGGCCTCTTGCTCTC
CGGCTCCCTCTGTTGCCCTCTGGTTTCTCCCCAGGC TCC CGG ACG TCC CTG CTC CTG GCT TTT GGC CTG CTC TGC CTG CCC TGG CTT
Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu Cys Leu Pro Trp Leu
+20 Asp of processed TBP1
CAA GAG GGC AGT GCC GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT TCG ATT TGC TGT ACC AAG TGC CAC AAA GGA
Gln Glu Gly Ser Ala Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys Gly
ACC TAC TTG TAC AAT GAC TGT CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TGT GAG AGC GGC TCC TTC ACC GCT TCA GAA AAC CAC CTC
Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
AGA CAC TGC CTC AGC TGC TCC AAA TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TCT TGC ACA GTG GAC CGG GAC ACC GTG TGT GGC TGC
Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys
AGG AAG AAC CAG TAC CGG CAT TAT TGG AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC AAT GGG ACC GTG CAC CTC TCC TGC
Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys
CAG GAG AAA CAG AAC ACC GTG TGC ACC TGC CAT GCA GGT TTC TTT CTA AGA GAA AAC GAG TGT GTC TCC TGT AGT AAC TGT AAG AAA AGC CTG
Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu
Linker
+7 Cys of hCG alpha
GAG TGC ACG AAG TTG TGC CTA CCC CAG ATT GAG AAT GTT AAG GGC ACT GAG GAC TCA GGC ACC ACA GCC GGT GCT GCC CCA GGT TGC CCA
Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser Gly Thr Thr Ala Gly Ala Ala Pro Gly Cys Pro
GAA TGC ACG CTA CAG GAA AAC CCA TTC TTC TCC CAG CCG GGT GCC CCA ATA CTT CAG TGC ATG GGC TGC TGC TTC TCT AGA GCA TAT CCC ACT
Glu Cys Thr Leu Gln Glu Asn Pro Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys Phe Ser Arg Ala Tyr Pro Thr
CCA CTA AGG TCC AAG AAG ACG ATG TTG GTC CAA AAG AAC GTC ACC TCA GAG TCC ACT TGC TGT GTA GCT AAA TCA TAT AAC AGG GTC ACA GTA
Pro Leu Arg Ser Lys Lys Thr Met Leu Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser Tyr Asn Arg Val Thr Val
ATG GGG GGT TTC AAA GTG GAG AAC CAC ACG GCG TGC CAC TGC AGT ACT TGT TAT TAT CAC AAA TCT TAA GGATCCCTCGAG
Met Gly Gly Phe Lys Val Glu Asn His Thr Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser ***
Bam HI XhoI

FIG. 2b(1)

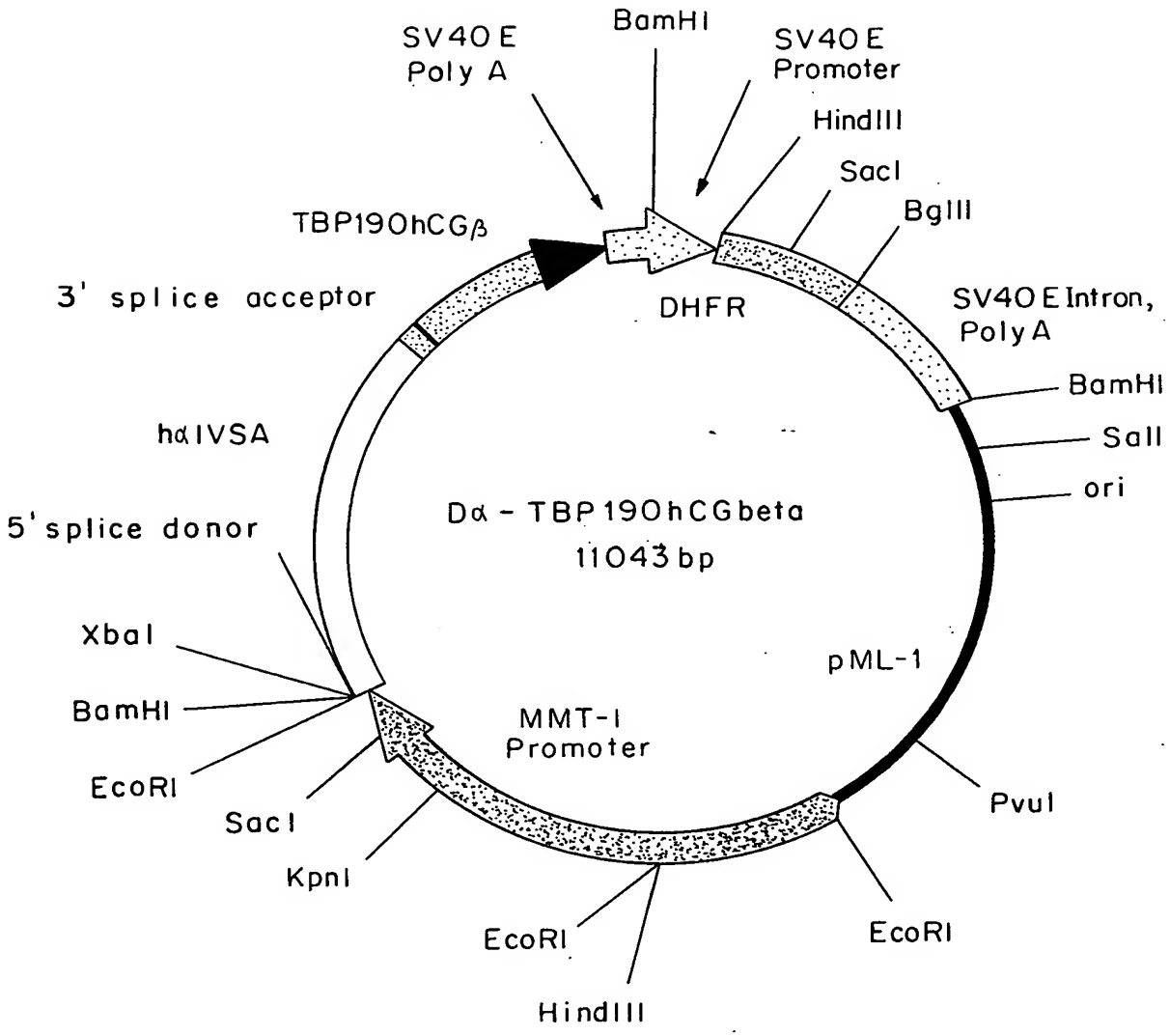

SV40 E
Poly A
BamHI
SV40 E
Promoter
HindIII
SacI
BglII
TBP190hCGβ
3' splice acceptor
DHFR
SV40 E Intron,
Poly A
BamHI
SalI
ori
hαIVSA
5' splice donor
Dα - TBP190hCGbeta
11043 bp
XbaI
BamHI
EcoRI
SacI
KpnI
MMT-1
Promoter
pML-1
PvuI
EcoRI
EcoRI
HindIII

FIG. 2b(2)

XhoI hGH Signal Sequence hGH Intron

CTCGAG ATG GCT ACA G GTAAGCGCCCCTAAAATCCCTTTGGGCACAATGTGTCCTGAGGGGAGAAGCAGCGACCTGTAGATGGGACGGGGGCACTAACCCTCAGGTTTGGG

▷Met Ala Thr

GCTTCTGAATGTGAGTATCGCCATGTAAGCCCAGTATTTGGCCAATCTCAGAAAGCTCCTGGTCCCTGGAGGGATGGAGAGAGAAAAACAAACAGCTCCTGTAGCAGGTAGAGTGCTGGC

CTCTTGCTCTCCGGCTCCCTCTGTTGCCCTCTGGTTTCTCCCCAGG C TCC CGG ACG TCC CTG CTC CTG GCT TTT GGC CTG CTC TGC CTG

▷Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu Cys Leu

+20 Asp of Processed TBP1

CCC TGG CTT CAA GAG GGC AGT GCC GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT TCG ATT TGC TGT ACC

▷Pro Trp Leu Gln Glu Gly Ser Ala Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr

AAG TGC CAC AAA GGA ACC TAC TTG TAC AAT GAC TGT CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TGT GAG AGC GGC TCC TTC ACC

▷Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr

GCT TCA GAA AAC CAC CTC AGA CAC TGC CTC AGC TGC TCC AAA TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TCT TGC ACA GTG GAC

▷Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp

CGG GAC ACC GTG TGT GGC TGC AGG AAG AAC CAG TAC CGG CAT TAT TGG AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC

▷Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu

AAT GGG ACC GTG CAC CTC TCC TGC CAG GAG AAA CAG AAC ACC GTG TGC ACC TGC CAT GCA GGT,TTC TTT CTA AGA GAA AAC GAG TGT GTC

▷Asn Gly Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val

TCC TGT AGT AAC TGT AAG AAA AGC CTG GAG TGC ACG AAG TTG TGC CTA CCC CAG ATT GAG AAT GTT AAG GGC ACT GAG GAC TCA GGC ACC

▷Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser Gly Thr

Linker +7 Pro of beta

ACA GCT GGT GCT GGT CCA CGG TGC CGC CCC ATC AAT GCC ACC CTG GCT GTG GAG AAG GAG GGC TGC CCC GTG TGC ATC ACC GTC AAC

▷Thr Ala Gly Ala Gly Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val Asn

ACC ACC ATC TGT GCC GGC TAC TGC CCC ACC ATG ACC CGC GTG CTG CAG GGG GTC CTG CCG GCC CTG CCT CAG GTG GTG TGC AAC TAC CGC

▷Thr Thr Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg

GAT GTG CGC TTC GAG TCC ATC CGG CTC CCT GGC TGC CCG CGC GGC GTG AAC CCC GTG GTC TCC TAC GCC GTG GCT CTC AGC TGT CAA TGT

▷Asp Val Arg Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys

GCA CTC TGC CGC CGC AGC ACC ACT GAC TGC GGG GGT CCC AAG GAC CAC CCC TTG ACC TGT GAT GAC CCC CGC TTC CAG GAC TCC TCT TCC

▷Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser Ser

TCA AAG GCC CCT CCC CCC AGC CTT CCA AGC CCA TCC CGA CTC CCG GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA TAA GGATCCCTCGAG

▷Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln *** BamHI XhoI

FIG. 3

p55 TNFR1, TBP1 and TBP1 FUSION CONSTRUCTS

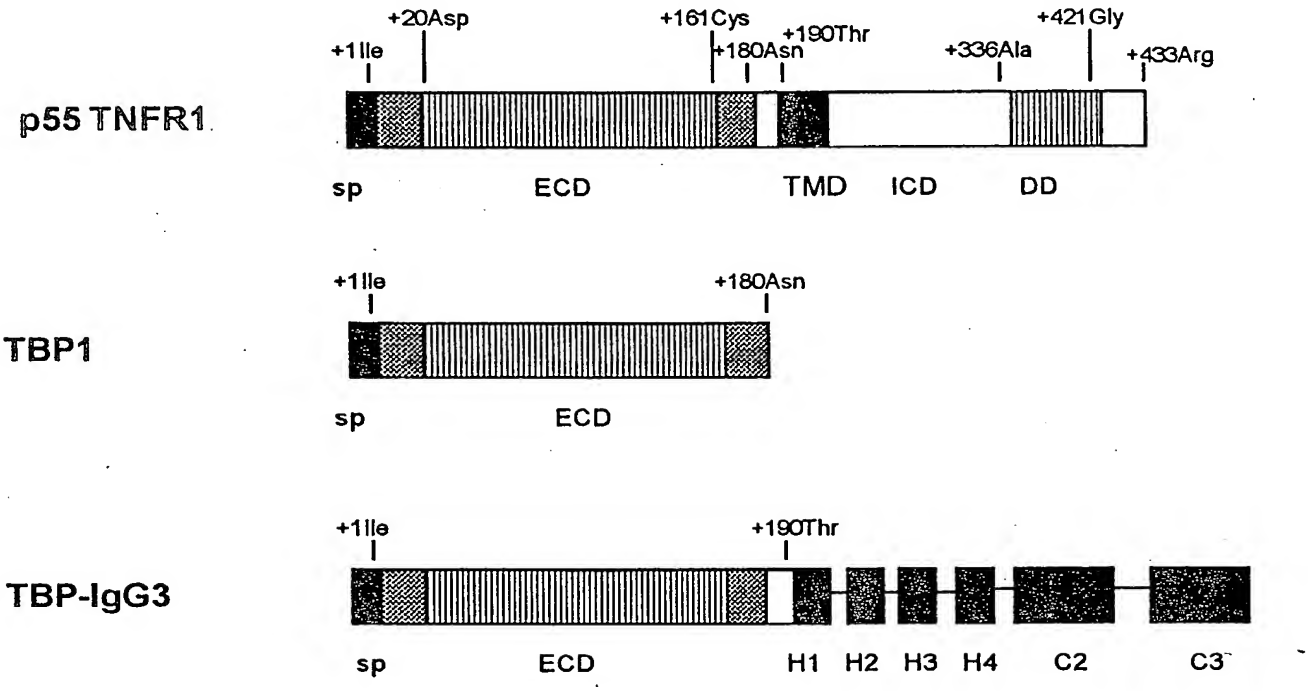


FIG. 4

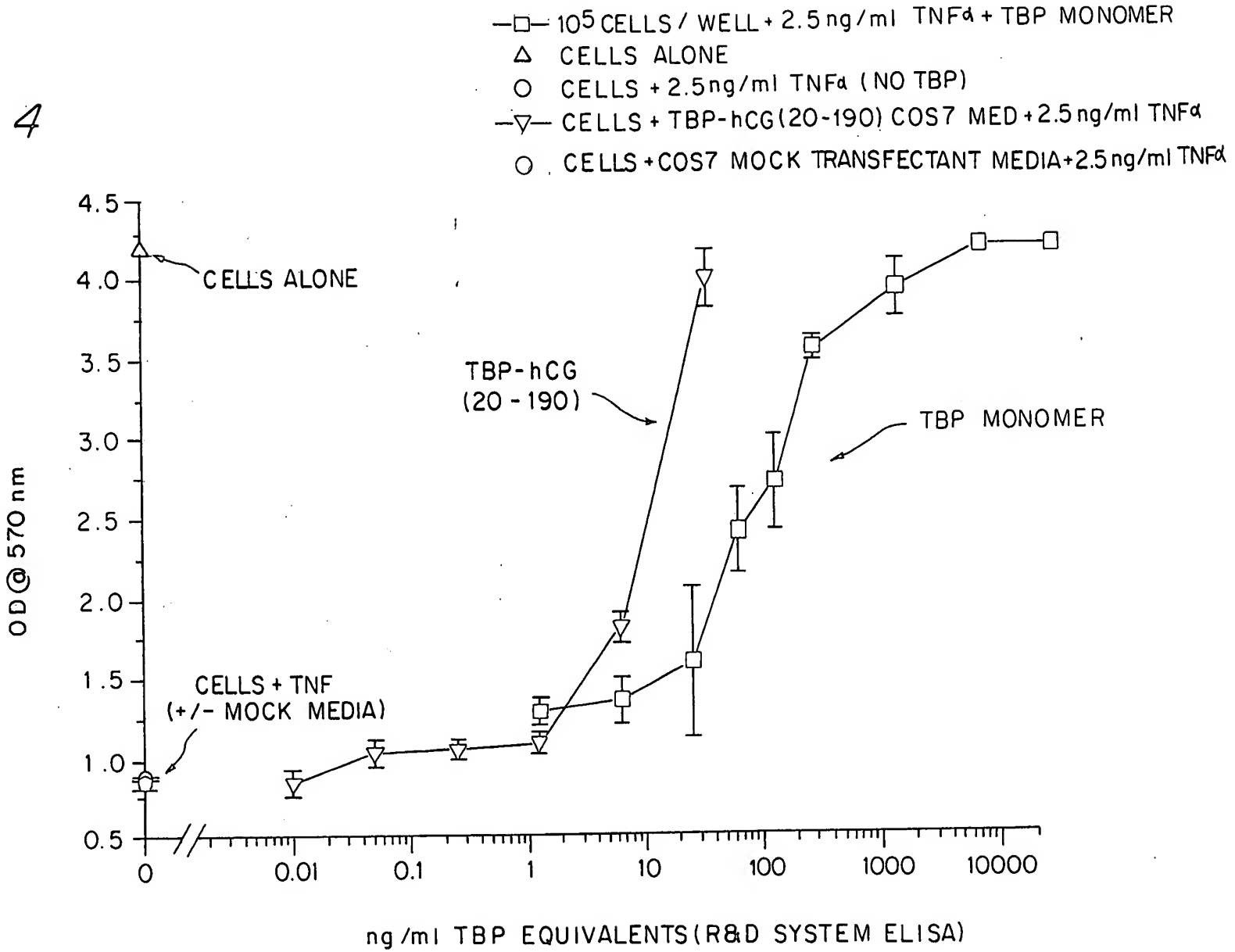

—□— 10^5 CELLS / WELL + 2.5 ng/ml TNFα + TBP MONOMER
△ CELLS ALONE
○ CELLS + 2.5 ng/ml TNFα (NO TBP)
—▽— CELLS + TBP-hCG (20-190) COS7 MED + 2.5 ng/ml TNFα
○ CELLS + COS7 MOCK TRANSFECTANT MEDIA + 2.5 ng/ml TNFα
OD @ 570 nm
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
CELLS ALONE
TBP-hCG (20-190)
TBP MONOMER
CELLS + TNF (+/- MOCK MEDIA)
0
0.01
0.1
1
10
100
1000
10000
ng/ml TBP EQUIVALENTS (R&D SYSTEM ELISA)

FIG. 5

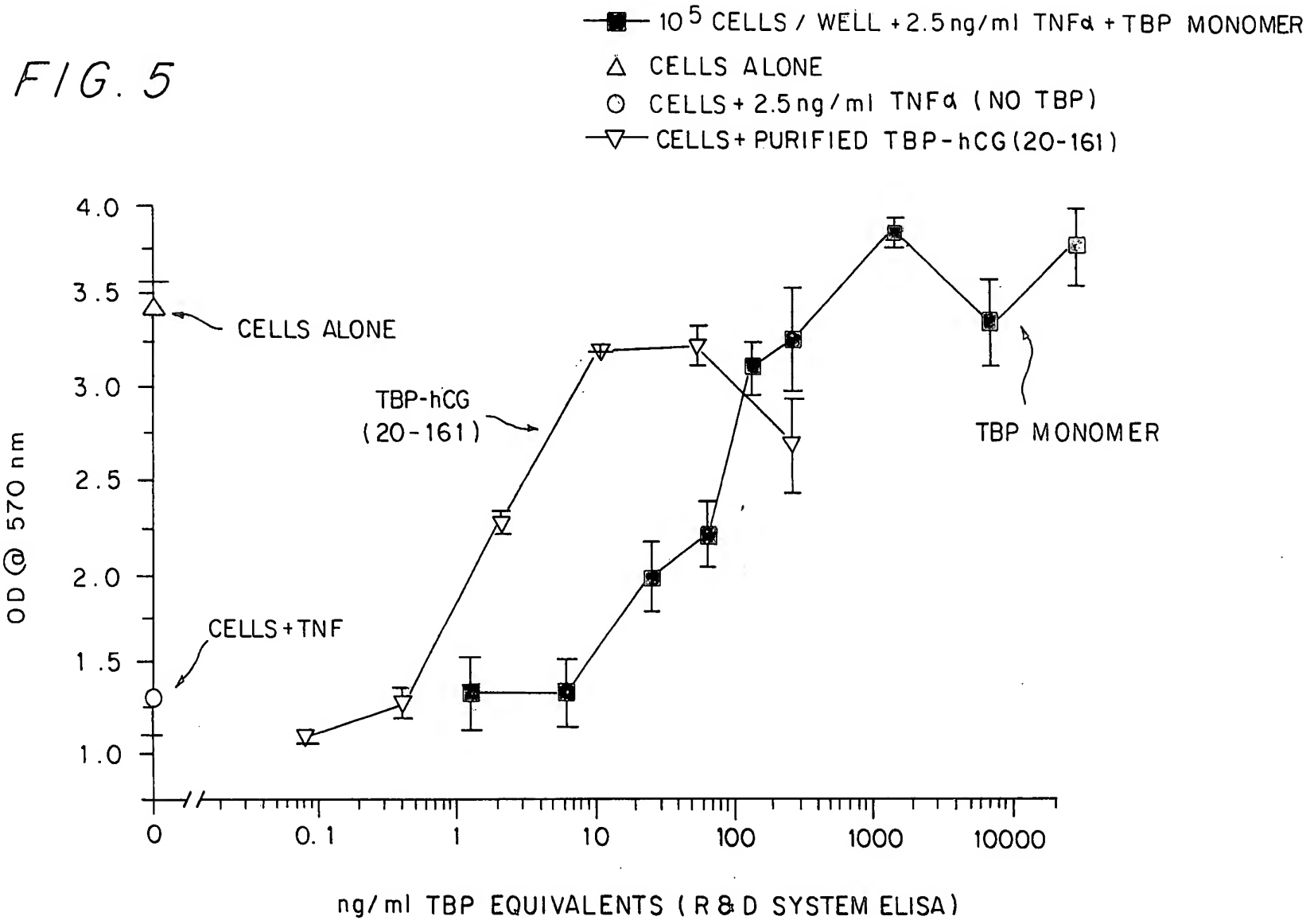
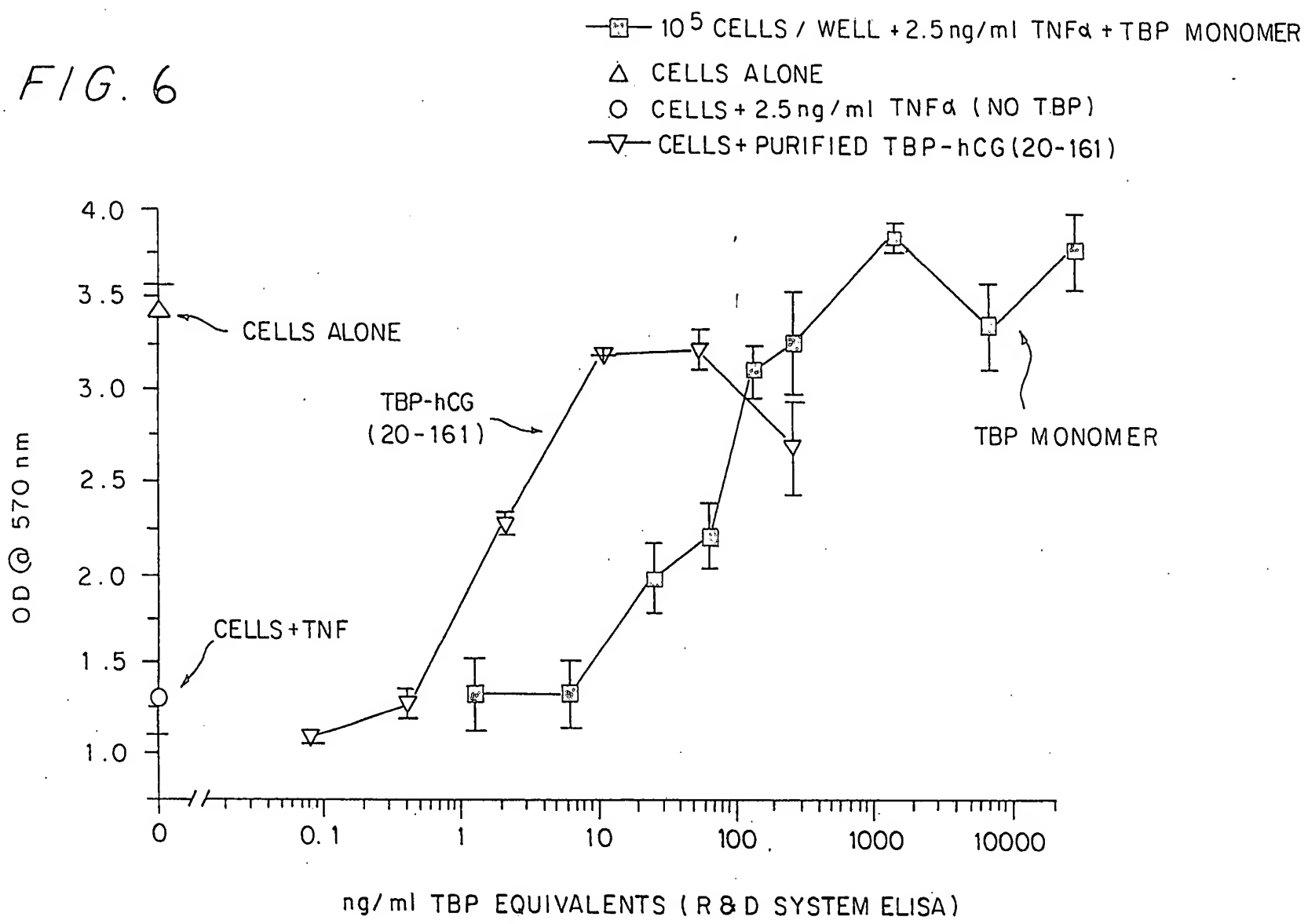

10^5 CELLS / WELL + 2.5 ng/ml TNFα + TBP MONOMER
CELLS ALONE
CELLS + 2.5 ng/ml TNFα (NO TBP)
CELLS + PURIFIED TBP-hCG (20-161)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
OD @ 570 nm
CELLS ALONE
TBP-hCG
(20-161)
TBP MONOMER
CELLS + TNF
0
0.1
1
10
100
1000
10000
ng/ml TBP EQUIVALENTS (R&D SYSTEM ELISA)

FIG. 6


10^5 CELLS / WELL + 2.5 ng/ml TNFα + TBP MONOMER
CELLS ALONE
CELLS + 2.5 ng/ml TNFα (NO TBP)
CELLS + PURIFIED TBP-hCG (20-161)
OD @ 570 nm
4.0
3.5
3.0
2.5
2.0
1.5
1.0
CELLS ALONE
TBP-hCG (20-161)
TBP MONOMER
CELLS + TNF
0
0.1
1
10
100
1000
10000
ng/ml TBP EQUIVALENTS (R & D SYSTEM ELISA)